ACS OMEGA

Open Access

http://pubs.acs.org/journal/acsodf

Article

Phlorizin Prolongs the Lifespan of *Caenorhabditis elegans* by Insulin and SIR-2.1 Regulation

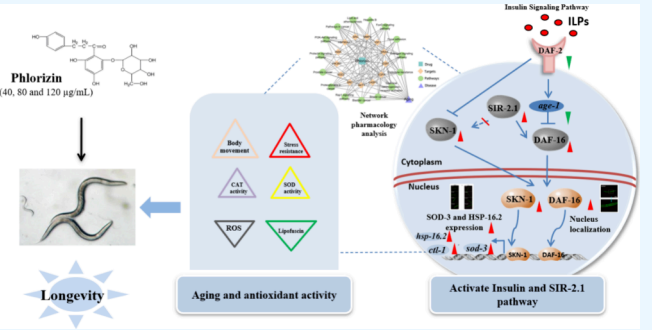
Xiaohan Zhang and Hao Wang*

Cite This: *ACS Omega* 2025, 10, 11922−11934

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Phlorizin has significant antioxidant properties and was studied using *Caenorhabditis elegans* to explore potential antiaging mechanisms. Results showed that phlorizin mitigated the harmful effects of high temperatures and hydrogen peroxide, reduced oxidative stress, increased antioxidant enzyme activity, and reduced malondialdehyde levels. Network pharmacological analysis reveals that the AKT1, INSR, and SOD2 signaling pathways play a key role in the antiaging effects of phlorizin. Its action is mediated by insulin and SIR-2.1, influencing DAF-16, SKN-1, and downstream genes in the antiaging effects. This implicates phlorizin as a promising functional food additive targeting the DAF-16 and SOD-3 axes for antiaging.



1. INTRODUCTION

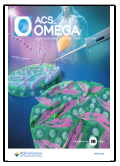
Aging is a multifaceted biological process characterized by a gradual decline in physiological functions and a weakening of the body's regulatory systems.[1] Central to this process is the delicate balance between the production of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms. When ROS levels escalate abnormally, they can trigger a cascade of detrimental effects, including necrosis, apoptosis, and the acceleration of the aging process in a variety of human disorders.[2] The body's response to oxidative stress is a testament to the complexity of cellular homeostasis,[3] where antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) play a vital role in neutralizing ROS and mitigating oxidative damage to living cells.[4]

The molecular mechanisms underpinning the aging process are intricate and involve several signaling pathways, among which the insulin and insulin-like growth factor (IGF1) pathway are two of the most well studied.[5,6] The activation of this pathway through the binding of insulin-like peptides to the receptor *daf-2* initiates a phosphorylation cascade that modulates the activity of the DAF-16 and FOXO transcription factors, a key regulator of longevity.[7] Recent studies have also highlighted the crosstalk between the IGF1 pathway and other stress response pathways, such as the heat shock factor (HSF) and the AMP-activated protein kinase (AMPK) pathways, which further modulate the aging process.[8,9]

Plants like grapes and grape seeds contain phlorizin, a chalcone compound that has emerged as a promising candidate in the quest to understand and potentially combat aging.[10] Its biological activities have been extensively studied, revealing its ability to extend the lifespan of yeast and *Drosophila melanogaster* through modulation of antioxidant enzymes and the Keap1-Nrf2 pathway.[11,12] Moreover, phlorizin's neuroprotective properties have been demonstrated in models of Alzheimer's disease and Parkinson's disease, where it has been shown to reduce oxidative stress and inflammation and protect against neuronal damage.[13−15] Recent advancements in the field have also explored the role of microRNAs and long noncoding RNAs in the regulation of aging, adding another layer of complexity to our understanding of the molecular mechanisms involved.[16,17] Furthermore, the burgeoning field of epigenetics has shed light on how modifications such as DNA methylation and histone acetylation can influence aging and the response to oxidative stress.[18,19]

This study aims to build upon the existing body of knowledge by investigating the effects of phlorizin on the lifespan of *C. elegans*, a widely used model organism in aging research. We explore the role of phlorizin in enhancing antioxidant enzyme activity, reducing free radical damage, and improving stress resistance. We will also use several mutant strains of *C. elegans* and the latest bioinformatics tools to break down the complicated pathways that phlorizin controls, giving us a full picture of how it fights aging.

Received: September 23, 2024
Revised: February 21, 2025
Accepted: March 5, 2025
Published: March 24, 2025



2. MATERIALS AND METHODS

2.1. Materials. Phlorizin (>96% purity) was sourced from Jianfeng Natural Product Co., Tianjin, China. Reagents including hydrogen peroxide, 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA), dimethyl sulfoxide (DMSO), and fluorodeoxyuridine (FUDR) were procured from Sigma-Aldrich, St. Louis, Michigan, USA. Assay kits for the determination of SOD and CAT activities, as well as malondialdehyde (MDA), were obtained from the Nanjing Jiancheng Bioengineering Institute. TRIzol reagent and SYBR Green kits were supplied by Thermo Fisher Scientific, Carlsbad, California, USA.

2.2. *C. elegans* Strains and Maintenance. The *C. elegans* strains were obtained from the Caenorhabditis Genetics Center (CGC). Strains were cultured on nematode growth medium (NGM) with a bacterial food source of inactivated *Escherichia coli* OP50, heat-killed at 65 °C for 30 min. The cultivation was conducted at a constant temperature of 20 °C. The strains utilized in this study included wild-type N2 and various mutant strains: TJ375 [hsp-16.2p::GFP], LD1 [skn-1b/c::GFP + rol-6(su1006)], CF1553 [sod-3p::GFP], DA1116 [*eat-2*(ad1116)], CF1038 [*daf-16*(mu86)], CB1370 [*daf-2*(e1370)], VC199 [*sir-2.1*(ok434)], EU1 [*skn-1*(zu67)], and TJ356 [daf-16p::DAF-16a/b::GFP; rol-6]. Phlorizin was prepared as a stock solution in DMSO at a final concentration not exceeding 0.1%, a concentration that has been shown to be nontoxic to *C. elegans*.[20]

2.3. Network Pharmacology Analysis. Initial target identification was performed using the Pharm Mapper and TCMSP databases followed by gene name normalization through the UniProt database. Genes implicated in aging were identified using Gene Cards. Intersection of phlorizin targets and aging-associated genes was determined with the Venny tool. The resulting gene list was analyzed for protein−protein interactions using the STRING database, filtered for human homologues. Subsequently, the PPI network was visualized and analyzed by using Cytoscape software. Functional enrichment analysis of the identified genes was conducted through the DAVID Bioinformatics Resources, focusing on the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and Gene Ontology (GO) terms. Finally, an integrated network illustrating the connections among drugs, targets, pathways, and diseases was constructed by using Cytoscape.

2.4. Lifespan Assay. Lifespan experiments were conducted on synchronized L4-stage *C. elegans* (both mutants and wild type) at 20 °C. Approximately 120 L4 larvae were placed on NGM agar plates, either treated with varying concentrations of phlorizin (0 to 200 μg/mL) or left untreated. The plates were supplemented with heat-inactivated *E. coli* OP50 and 150 mmol/L of 5-fluoro-2′-deoxyuridine (FUdR) to prevent progeny production. To avoid the loss of worms due to desiccation or escape, *E. coli* OP50 was uniformly spread on the NGM surface, avoiding the edges.

Survival of the worms was monitored every 48 h until all had died. Lifespan data were analyzed using Prism 5 software, excluding individuals with non-normal deaths such as those caused by dissection, loss from the culture plate, or desiccation.[21] Each experiment was replicated a minimum of three times to ensure the reliability of the results.

2.5. Pharynx Pumping and Body Movement Analysis. The pharynx pumping assay and body movement analysis were performed as previously described.[22] *C. elegans* were cultured on NGM plates, either supplemented with phlorizin or left untreated as a control. Pharyngeal pumping activity was measured under a stereomicroscope for 30 s on days 2, 4, 6, 8, and 10 postinoculation. For each condition, 60 nematodes were selected from three independent replicates.

Body movement was assessed by gently stimulating the nematodes with a needle every 48 h. Nematodes exhibiting continuous and consistent sinusoidal movement within 30 s were categorized as having fast motion; those with less frequent or irregular movement were categorized as having slow motion.

2.6. Oxidative Stress Assay. We conducted oxidative stress assays with minor modifications to established methods.[23] For the thermal stress assay, 120 *C. elegans* were exposed to a heat shock at 30 °C for 1 h and then shifted to a more severe stress at 36 °C. Survival rates were monitored hourly until all worms had died. In the acute oxidative stress assay, 120 worms were treated with hydrogen peroxide (30 mM final concentration in 1 mL of NGM culture medium). Mortality was recorded at daily intervals or every 30 min, depending on the rate of death observed, until the last worm died. Each assay was performed in triplicate to ensure the reproducibility of the results.

2.7. Measurement of Endogenous ROS and Blue Autofluorescence. We assessed endogenous ROS and blue autofluorescence in *C. elegans* using established methods with minor adjustments.[24] Worms were cultured on NGM plates, with or without phlorizin, at 20 °C for their average lifespan. For ROS detection, worms were washed in phosphate-buffered saline (PBS) and incubated with 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) in the dark for 20 min. ROS fluorescence was measured using a fluorescence microscope (excitation at 485 nm, emission at 530 nm, 10× magnification).

Blue autofluorescence levels were determined by immobilizing worms with sodium azide (NaN_3) on 1% agarose-coated glass slides. Fluorescence was observed under the same microscope (excitation at 340−380 nm, emission at 430 nm, 10× magnification). For each condition, 50 worms were randomly selected for quantification.

2.8. Nuclear Translocation of DAF-16 and SKN-1, and Expression Analysis of SOD-3::GFP and HSP-16.2::GFP. Transgenic *C. elegans* strains TJ356, LD1, CF1553, and TJ375 were cultivated on NGM with or without phlorizin for 72 h at 20 °C. After cultivation, worms were washed to remove *E. coli* OP50. The worms were then transferred onto slides coated with 1% agar and imaged using an inverted phase fluorescence microscope (Olympus cxk41) to visualize the nuclear localization of DAF-16::GFP and SKN-1::GFP fusion proteins, as well as the expression levels of HSP-16.2::GFP and SOD-3::GFP. Fluorescence was detected with excitation at 460−495 nm and emission at 510−550 nm, using a 10-objective lens. For the quantitative analysis, 50 worms were randomly selected from each group. This selection process was repeated for statistical analysis, and each experiment was independently performed at least three times to ensure the accuracy and reproducibility of the results.

2.9. Real-Time Quantitative PCR (qRT-PCR) Assay. Synchronized L4-stage *C. elegans* were cultured on NGM plates with or without phlorizin for 1 week. Subsequently, the worms were washed with PBS, and total RNA was extracted from approximately 2000 worms using the TRIzol reagent. Complementary DNA (cDNA) was synthesized from the

https://doi.org/10.1021/acsomega.4c08725
ACS Omega 2025, 10, 11922−11934

Table 1. PCR Primers Were Utilized to Estimate Gene mRNA Level in *C. elegans*

gene	forward primer 5′−3′	reverse primer 5′−3′
age-1	CCTGAACCGACTGCCAATC	GTGCTTGACGAGATATGTGTATT
sod-3	GGCTAAGGATGGTGGAGAAC	ACAGGTGGCGATCTTCAAG
daf-2	GCGGATACACAGCAAGAATAAC	GAGCCACAAGCACCAGAAC
sir-2.1	ACTGAGATGCTCCATGACAATAAG	GCAAGACGAACCACACGAAC
daf-16	TCAAGCCAATGCCACTACC	TGGAAGAGCCGATGAAGAAG
ctl-1	CTCCTCGGATTCTCTATCGGTATG	AAGGCGGCATCGGCAATG
act-1	CCAGGAATTGCTGATCGTATGCAGAA	TGGAGAGGGAAGCGAGGATAGA
skn-1	CACTGTCTCCTCTCATCATTGG	GAGTGTCTCTGTGAGTGATATGG
hsp-16.2	CTCAACGTTCCGTTTTGGT	CGTTGAGATTGATGGCAAAC
akt-1	TTCGAGCAAAGCCTAAGGA	ATAACATTGTCGCTGCATCG
eat-2	AACTTCGCCTGATCCTCCAA	ACCATATGCACAGGGAAGCT

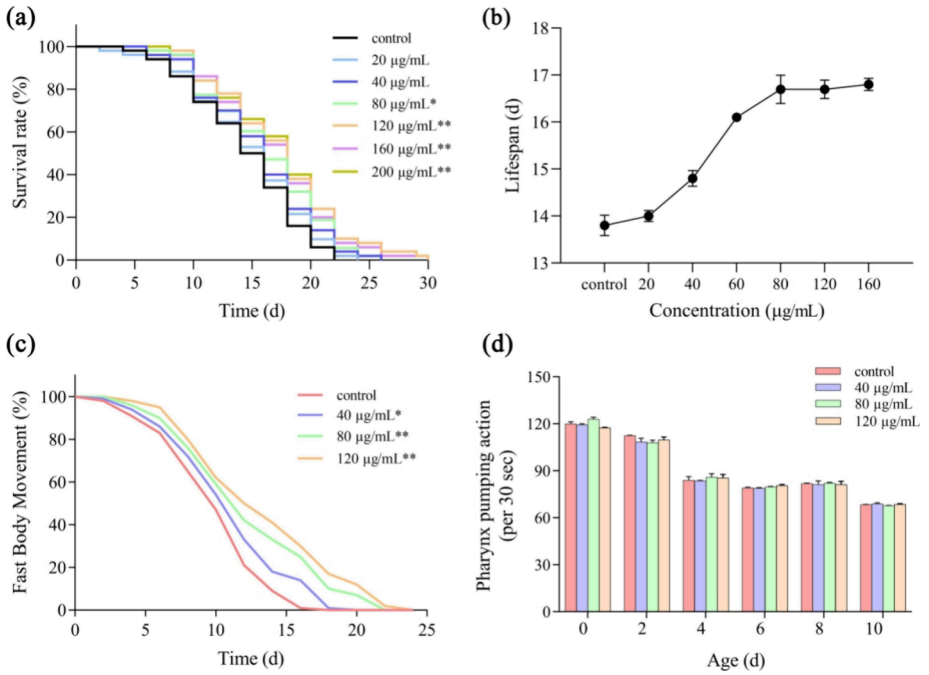


Figure 1. Effect of *C. elegans* exposed to different doses of phlorizin on the lifespan (a). Median lifespan (b). Age-related movement (c). Pharynx pumping (d). *$p < 0.05$ and **$p < 0.01$.

isolated RNA using a Quantscript RT Kit. qRT-PCR was performed using the Applied Biosystems Plus Real-Time PCR System with an SYBR Green PCR Master Mix (Applied Biosystems, Carlsbad, California, USA). PCR amplification was conducted under conditions previously reported.[25] Primer sequences are detailed in Table 1. The housekeeping gene act-1 was used as an endogenous control, and relative gene expression levels were determined using the comparative $2^{-\Delta\Delta Ct}$ method.

2.10. Enzymatic Activity Assay for SOD and CAT, and MDA Content Measurement. *C. elegans* cultivation followed the same conditions as for the lifespan assay. Approximately 2000 worms were washed with PBS and treated with 200 μL of sterile saline on ice. After centrifugation, the supernatant was collected. The homogenate of *C. elegans* was prepared following the manufacturer's protocol, and the activities of SOD and CAT, as well as MDA content, were measured using respective assay kits according to the provided instructions. Each assay was performed in triplicate to ensure accuracy and reproducibility of the results.

2.11. Molecular Coupling Analysis. Molecular docking was employed to investigate the interactions between phlorizin and key proteins. The structure of phlorizin was retrieved from the PubChem Database (https://pubchem.ncbi.nlm.nih.gov), while the three-dimensional structure of SOD-3 (PDB ID: 3DC5) was obtained from the RCSB Protein Data Bank. Additionally, the amino acid sequence for DAF-16 (Uni-ProKB-O16850) was sourced from the UNIPROT Database (http://uniprot.org). Prior to docking, both the receptor and the ligand were prepared using standard protocols. The molecular docking process was conducted using CDOCKER within the Discover Studio 3.5 software suite (Accelrys, California, USA).

2.12. Data Analysis. Statistical analyses were performed using Prism 5 software, and the results were expressed as the mean ± standard deviation (SD). Significant differences between groups were analyzed by a log-rank test in Kaplan−Meier survival analysis using SPSS 17.0 (Statistical Software for the Social Sciences, SPSS Inc.). Differences between means were assessed using one-way ANOVA. A p-value less than 0.05 indicated a significant difference, while p-values less than 0.01 indicated a highly significant difference. In addition, fluorescence intensity was measured using ImageJ software,

https://doi.org/10.1021/acsomega.4c08725
ACS Omega 2025, 10, 11922−11934

and body movement data was assessed using the ratio of the number of rapid movements.

3. RESULTS

3.1. Phlorizin Extended Survival and Enhanced Body Movement in *C. elegans*. We observed that the lifespan of *C. elegans* was extended with increasing doses of phlorizin. When phlorizin was added at 40, 80, and 120 μg/mL, the animals lived an average of 15.56 ± 2.08 days (7.76% longer, not significantly higher), 16.15 ± 1.30 days (11.84% longer, $p < 0.05$), and 17.30 ± 2.01 days (19.81% longer, $p < 0.01$), compared to the control group, which lived an average of 14.44 ± 1.60 days (Figure 1a and Table 2). Based on the median lifetime curve, we chose 40, 80, and 120 μg/mL concentrations for the late experiments (Figure 1b).

Table 2. Group of GO Projects Associated with Phlorizin

subgroup	GO term	count
biological process (BP)	negative regulation of the apoptotic process	7
	signal transduction	6
	positive regulation of protein phosphorylation	5
	positive regulation of transcription, DNA-templated	5
	positive regulation of ERK1 and ERK2 cascade	4
	positive regulation of cell migration	4
	negative regulation of intrinsic apoptotic signaling pathway	3
	positive regulation of cyclin-dependent protein serine/threonine kinase activity	3
	cellular response to ROS	3
	cellular response to cadmium ion	3
cellular component (CC)	cytoplasm	8
	nucleoplasm	6
	plasma membrane	6
	macromolecular complex	5
	perinuclear region of cytoplasm	4
	extracellular space	4
	membrane raft	3
	platelet alpha granule lumen	2
	extrinsic component of cytoplasmic side of plasma membrane	2
	ruffle membrane	2
molecular function (MF)	identical protein binding	6
	nitric oxide synthase regulator activity	4
	enzyme binding	4
	ATPase binding	3
	integrin binding	3
	ubiquitin protein ligase binding	3
	protein kinase activity	3
	protein serine/threonine/tyrosine kinase activity	3
	insulin receptor binding	2
	estrogen receptor binding	2

Aging in *C. elegans* is typically associated with a decline in body motility.[26] Our study found that phlorizin intervention significantly improved motility, with treatments at 80 and 120 μg/mL resulting in increases of 23.40% ($p < 0.05$) and 32.88% ($p < 0.05$), respectively, suggesting an enhancement in self-regulatory capacity (Figure 1c). The pharyngeal pumping rate was also measured to ensure that changes in body movement were not confounded by variations in food intake (Figure 1d).

3.2. Phlorizin Enhanced Stress Resistance in *C. elegans*. We assessed the impact of phlorizin on the stress resistance of *C. elegans* by measuring survival rates under hydrogen peroxide and heat stress conditions. Following exposure to hydrogen peroxide, *C. elegans* exhibited a rapid decline in viability, with the majority dying within 4 h. Treatment with phlorizin at concentrations of 40, 80, and 120 μg/mL resulted in average lifespan increases of 27.74% (n.s.), 40.65% ($p < 0.05$), and 57.43% ($p < 0.01$), respectively. Notably, the most significant enhancement in survival rate was observed between 2 and 3 h post-treatment (Figure 2a). These findings suggest that phlorizin may act as a protective agent against oxidative stress induced by hydrogen peroxide.

In the heat stress experiment, phlorizin intervention for 1 h did not significantly improve the immediate survival of *C. elegans*. However, when treated with phlorizin concentrations of 80−120 μg/mL for durations of 4−8 h, the lifespan was extended. Specifically, at a dose of 120 μg/mL, the lifespan increase reached 37.44% ($p < 0.01$) (Figure 2b). These results indicate that phlorizin could potentially protect against heat-induced oxidative damage.

3.3. Phlorizin Enhanced Antioxidant Defenses in *C. elegans*. This study evaluated the impact of phlorizin on biomarkers of aging in *C. elegans* at the 10-day stage, a period characterized by significant increases in aging indices. We focused on the effects of phlorizin (40, 80, and 120 μg/mL) on ROS and blue autofluorescence levels. The treatment significantly reduced ROS levels by 18.31% ($p < 0.05$), 24.46% ($p < 0.01$), and 36.63% ($p < 0.01$) (Figure 2c). Blue autofluorescence decreased by 21.74% ($p < 0.05$), 30.43% ($p < 0.05$), and 50.00% ($p < 0.01$) (Figure 2d and Table S3).

Analysis of antioxidant enzymes SOD and CAT, along with MDA levels, confirmed phlorizin's (40, 80, and 120 μg/mL) antioxidant potency. The results showed that SOD activity increased by 3.99% ($p < 0.01$), 7.63%, and 18.94%; CAT activity increased by 4.20% ($p > 0.05$), 6.72% ($p < 0.01$), and 31.09%, respectively (Figure 2e); and the MDA content decreased by 1.58% ($p > 0.05$), 15.53% ($p < 0.05$), 17.71% ($p < 0.05$), and 17.71% ($p < 0.01$) (Figure 2f). Phlorizin significantly bolstered *C. elegans*' antioxidant defenses, reducing ROS and MDA while elevating SOD and CAT activities, suggesting its potential to combat oxidative stress and extend healthspan.

3.4. Network Pharmacology Analysis of Phlorizin-Associated Targets and Pathways. The protein−protein interaction (PPI) network diagram in Figure 3a illustrates the complex interactions among 115 targets linked to both phlorizin and aging. Notably, AKT1, INSR, and SOD2 were identified as pivotal targets for phlorizin, as detailed in the Supporting Information. Figure 3b and Table 2 present the Gene Ontology (GO) annotations, which were utilized to elucidate the functional roles of these targets. The analysis categorized the biological processes (BP), cellular components (CC), and molecular functions (MF) of the target proteins, revealing significant enrichment in negative regulation of apoptotic processes and signal transduction for BP, a concentration in the cytoplasm, nucleoplasm, and plasma membrane for CC, and prominent roles in identical protein interaction, regulator activity of nitric oxide synthase, and enzyme binding for MF.

The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment results are shown in Figure 3c and Table 3. These showed the top 15 pathways that were linked to phlorizin. The FoxO signaling pathway was highlighted as one of the top three pathways, indicating its potential importance.

https://doi.org/10.1021/acsomega.4c08725
ACS Omega 2025, 10, 11922−11934

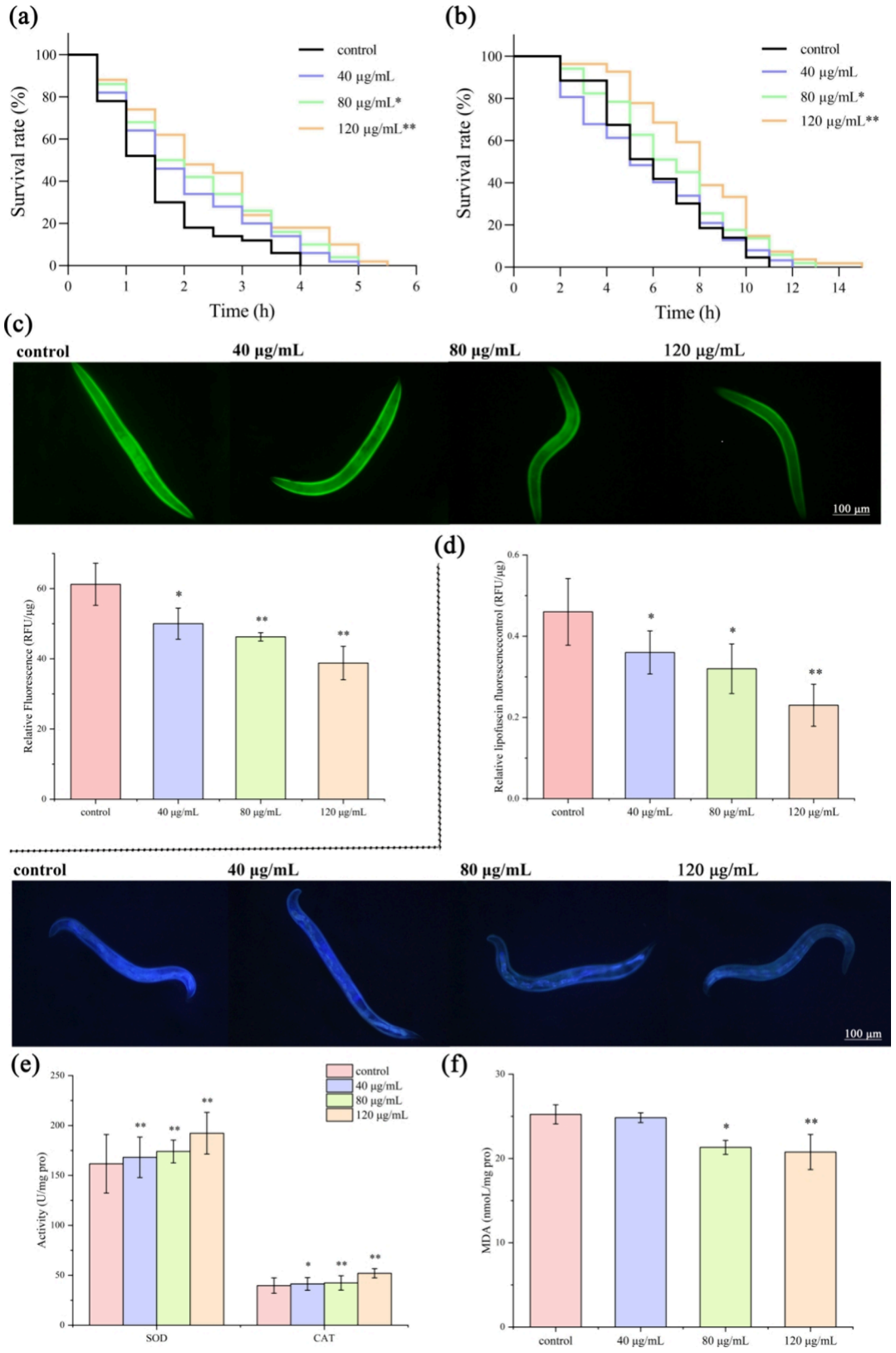


Figure 2. Effect of phlorizin on the antioxidant ability of *C. elegans*. (a) Hydrogen peroxide. (b) High temperature (36 °C). Representative images and fluorescence quantification of ROS (c) and blue autofluorescence (d) in wild type with or without phlorizin supplementation. Effect of phlorizin on SOD and CAT activities (e) and MDA content (f) in wild-type N_2. *$p < 0.05$ and **$p < 0.01$.

Figure 3d depicts the relationship between the common targets and the top 15 KEGG pathways, providing a visual network diagram that integrates ″phlorizin-target-pathway-aging″ correlations. This analysis suggests that phlorizin may modulate multiple targets and pathways, thereby exerting its effects on aging.

https://doi.org/10.1021/acsomega.4c08725
ACS Omega 2025, 10, 11922−11934

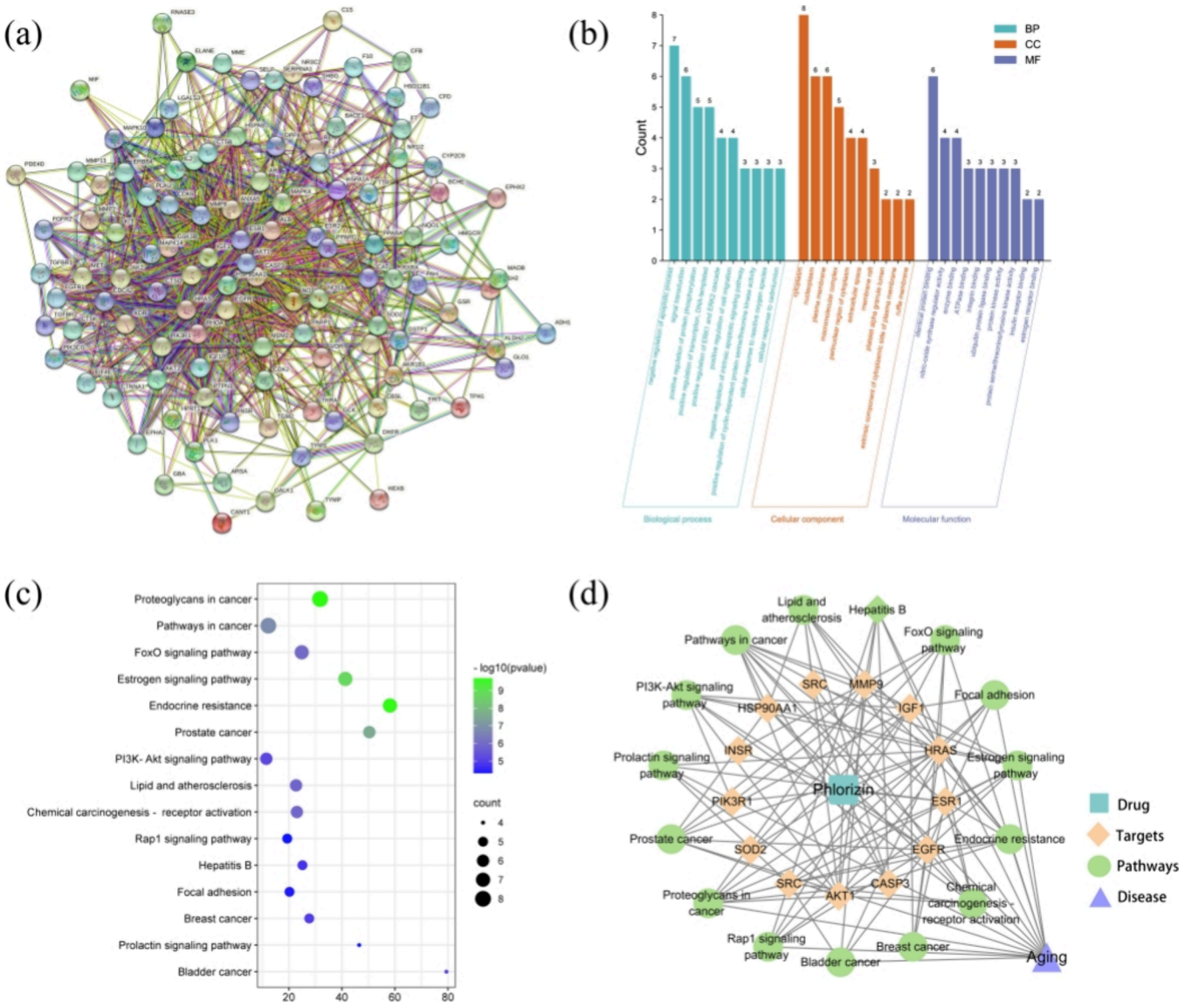


Figure 3. Network pharmacological analysis of phlorizin. (a) PPI construction plot. (b) Three classifications of GO items were acquired from DAVID databases, including BP, MF, and CC. (c) The top 15 KEGG pathways were ranked based on the quantity of genes linked to phlorizin. (d) Connections between the common targets of phlorizin and the leading 15 KEGG pathways.

3.5. Phlorizin Modulates Longevity via SIR-2.1 and IIS Pathways. It can be seen in Figure 4a that 120 μg/mL phlorizin decreased the expression of *daf-2*, *age-1*, and *akt-1* while increasing the expression of *daf-16*, *skn-1*, and *sir-2.1*, and antioxidant genes *hsp-16.2*, *sod-3*, and *ctl-1*. It had no effect on *eat-2* (Table S1). This suggests that phlorizin's action may not involve dietary restriction pathways. Lifespan studies in mutants treated with 120 μg/mL phlorizin revealed no significant changes in *daf-2* (Figure 4b), *daf-16* (Figure 4c), *skn-1* (Figure 4d), and *sir-2.1* mutants (Figure 4e), indicating their importance in lifespan regulation (Table 4). In contrast, *eat-2* mutants showed a 13.56% increase in longevity, suggesting *eat-2*'s minor role in phlorizin's effect on lifespan (Figure 4f).

The impact of phlorizin on ROS and blue autofluorescence regulation was examined in mutants, with no significant difference observed compared to controls (Figure 4g,h). Antioxidant enzyme activities SOD and CAT increased marginally after phlorizin treatment in mutants, without reaching significance, and MDA content trends were non-significant (Figure 4i,j). These findings highlight the complexity of phlorizin's metabolic pathways and confirm the indispensable roles of these genes in phlorizin's regulation of antioxidant effects and lifespan in *C. elegans*.

3.6. Phlorizin Promoted the Nuclear Translocation of the DAF-16 and SKN-1 Proteins. Phlorizin enhanced the nuclear translocation of DAF-16 and SKN-1 proteins in transgenic *C. elegans* strains LG333 SKN-1::GFP and TJ356 DAF-16::GFP. Figure 5a illustrates SKN-1::GFP distribution, with the nucleus fraction increasing from 10.43 ± 2.16% in controls to 18.54 ± 3.60% with 120 μg/mL phlorizin ($p < 0.05$). Similarly, DAF-16::GFP translocation to the nucleus was significantly elevated by phlorizin treatment, with the nuclear fraction rising from 3.82 ± 1.67% in controls to 15.43 ± 2.32% at the highest phlorizin concentration ($p < 0.01$, Figure 5b). These findings indicate that phlorizin may extend *C. elegans* longevity by promoting SKN-1 and DAF-16 nuclear translocation.

https://doi.org/10.1021/acsomega.4c08725
ACS Omega 2025, 10, 11922−11934

Table 3. Enrichment of the KEGG

term	count	target
proteoglycans in cancer	8	SRC, CASP3, AKT1, IGF1, HRAS, ESR1, MMP9, EGFR
endocrine resistance	7	SRC, AKT1, IGF1, HRAS, ESR1, MMP9, EGFR
estrogen signaling pathway	7	HSP90AA1, SRC, AKT1, HRAS, ESR1, MMP9, EGFR
prostate cancer	6	HSP90AA1, AKT1, IGF1, HRAS, MMP9
pathways in cancer	7	CASP3, AKT1, IGF1, HRAS, ESR1, MMP9
FoxO signaling pathway	7	AKT1, IGF1, HRAS, EGFR, SOD2, PIK3R1, INSR
chemical carcinogenesis-receptor activation	6	HSP90AA1, SRC, AKT1, HRAS, ESR1, EGFR
lipid and atherosclerosis	6	HSP90AA1, SRC, CASP3, AKT1, HRAS, MMP9
PI3K-Akt signaling pathway	6	HSP90AA1, AKT1, IGF1, HRAS, EGFR, PIK3R1
bladder cancer	9	SRC, HRAS, MMP9, EGFR,AKT1, IGF1, HRAS, ESR1, EGFR
hepatitis B	5	SRC, CASP3, AKT1, HRAS, MMP9
focal adhesion	5	SRC, AKT1, IGF1, HRAS, EGFR
Rap1 signaling pathway	5	SRC, AKT1, IGF1, HRAS,EGFR
prolactin signaling pathway	4	SRC, AKT1, HRAS, ESR1

3.7. Phlorizin Enhanced the Expression of *daf-16* Downstream Genes. We examined the influence of phlorizin on the expression of *daf-16* and its downstream genes in *C. elegans*. Figure 6a shows that phlorizin treatment did not significantly alter *daf-16* mRNA levels in mutants CB1370 *daf-2* (e1370) (1.12 ± 0.18-fold, $p < 0.05$) and VC199 *sir-2.1* (*ok434*) (1.18 ± 0.26-fold, $p < 0.05$). Similarly, *skn-1* mRNA expression in these mutants was not significantly increased in CB1370 *daf-2* (*e1370*) (1.46 ± 0.08-fold, $p < 0.05$), but a significant enhancement was observed in VC199 *sir-2.1* (*ok434*) (1.50 ± 0.14-fold, $p < 0.05$) upon treatment with 120 μg/mL phlorizin (Figure 6b). The expression of *daf-16* downstream genes *sod-3*, *ctl-1*, and *hsp-16.2* was also assessed in mutant CF1038 *daf-16* (*mu86*). However, no significant increase was detected in the expression of *sod-3* (1.08 ± 0.11-fold, $p > 0.05$), *ctl-1* (1.28 ± 0.21-fold, $p > 0.05$), or *hsp-16.2* (1.13 ± 0.16-fold, $p > 0.05$) (Figure 6c).

To further investigate the effects of phlorizin on the expression of antioxidant proteins, we used transgenic nematodes CF1553 [(pAD76) sod-3p::GFP] and TJ375 (gpIs1 [hsp-16.2p::GFP]). Phlorizin significantly enhanced SOD-3::GFP fluorescence at concentrations of 40 μg/mL (23.59%, $p < 0.05$), 80 μg/mL (46.14%, $p < 0.01$), and 120 μg/mL (102.61%, $p < 0.01$) (Figure 6d). HSP-16.2::GFP fluorescence was also upregulated by phlorizin at 80 μg/mL (41.59%; $p < 0.01$) and 120 μg/mL (107.03%; $p < 0.01$), with a nonsignificant increase at 40 μg/mL (6.29%; $p > 0.05$) (Figure 6e). The results indicate that phlorizin modulates the expression of antioxidant genes in a concentration-dependent manner. While mRNA levels of *daf-16* and *skn-1* did not show consistent changes across mutants, the significant upregulation of SOD-3::GFP and HSP-16.2::GFP fluorescence in response to phlorizin suggests that these proteins may contribute to the antioxidant effects of phlorizin. This modulation of antioxidant defenses could be a key mechanism by which phlorizin promotes longevity in *C. elegans*.

3.8. Molecular Coupling Analysis of Phlorizin Interaction with Aging-Related Proteins. Molecular coupling analysis was conducted to elucidate the interaction between phlorizin and the proteins implicated in aging. In order to elucidate the molecular basis of the action of phlorizin, the binding modes of phlorizin with DAF-16 and SOD-3 were analyzed. The analyses showed that phlorizin had a strong binding affinity for (−184.619 kJ/mol) (Figure 6f) and SOD-3 (−17.6047 kJ/mol) (Figure 6g), with a particularly significant interaction with DAF-16, in which the two-dimensional profile of the interaction showed the formation of hydrogen bonds with the participation of specific amino acid residues ASP258, ASN42, SER45, TYR127, and LYS122 (Table 5). The presence of these multiple hydrogen bonds suggests a strong binding capacity between phlorizin and these target proteins, thus potentially enhancing the strength of their interactions with the proteins. Through molecular docking techniques, key binding sites and types of interactions between phlorizin and these proteins were identified to better understand the binding mechanisms.

4. DISCUSSION

Aging is inevitable, but slowing it is possible. Dihydrochalcone has higher oxidation resistance than its corresponding brass due to its unique structure. Phlorizin belongs to the chalcone class of organic compounds, was originally isolated from the bark of apple trees, and has been used in human medicines to benefit from its biological activities. Our research looks at how phlorizin affects the longevity of *C. elegans* in great detail, showing that it might be useful as a medicine to fight the effects of getting older. The results show that phlorizin greatly lessens the harmful effects of H_2O_2 and high temperatures on the longevity of *C. elegans*. This is an especially important finding given the growing interest in figuring out the molecular causes of aging.

Aging encompasses a complex interplay of biological processes, fundamentally characterized by a reduction in functional capacity and an increased vulnerability to stress.[27] Our research into how phlorizin affects the aging process in *C. elegans* has given us interesting ideas about how it might be used to make people live longer. Phlorizin's ability to extend the lifespan of *C. elegans* in the absence of feeding level interference, as depicted in Figure 1, is a testament to its direct impact on aging mechanisms. The detrimental effects of oxidative stress, arising from the accumulation of ROS and blue autofluorescence, are well-established contributors to cellular senescence and the aging phenotype.[28,29] Blue autofluorescence, as a tryptophan-derived glucose ester of *o*-aminobenzoic acid, can be observed in nematode intestinal lysosome-associated organelles (intestinal granules) and correlates with organismal death.[28] In senescent *C. elegans*, an increase in blue fluorescence can be observed in intestinal cells due to cell necrosis.[30] Blue autofluorescence is a self "age pigment" produced by oxidative denaturation and autophagy of cellular components, and its accumulation in nematodes is only relevant to nematode lifespan, which is a recognized marker of nematode senescence.[31,32] The fluorescent substance has been found to be strongly associated with organismal senescence and reactive oxygen stress.[33] In this study, blue autofluorescence was used as a measure of senescence retardation of nematodes by phlorizin. Our study reveals that phlorizin significantly attenuates these effects, as evidenced by the diminished accumulation of ROS (Figure 2c and Table S2) and blue autofluorescence (Figure 2d and Table S3) in treated worms. This reduction is particularly significant, given the

https://doi.org/10.1021/acsomega.4c08725
ACS Omega 2025, 10, 11922−11934

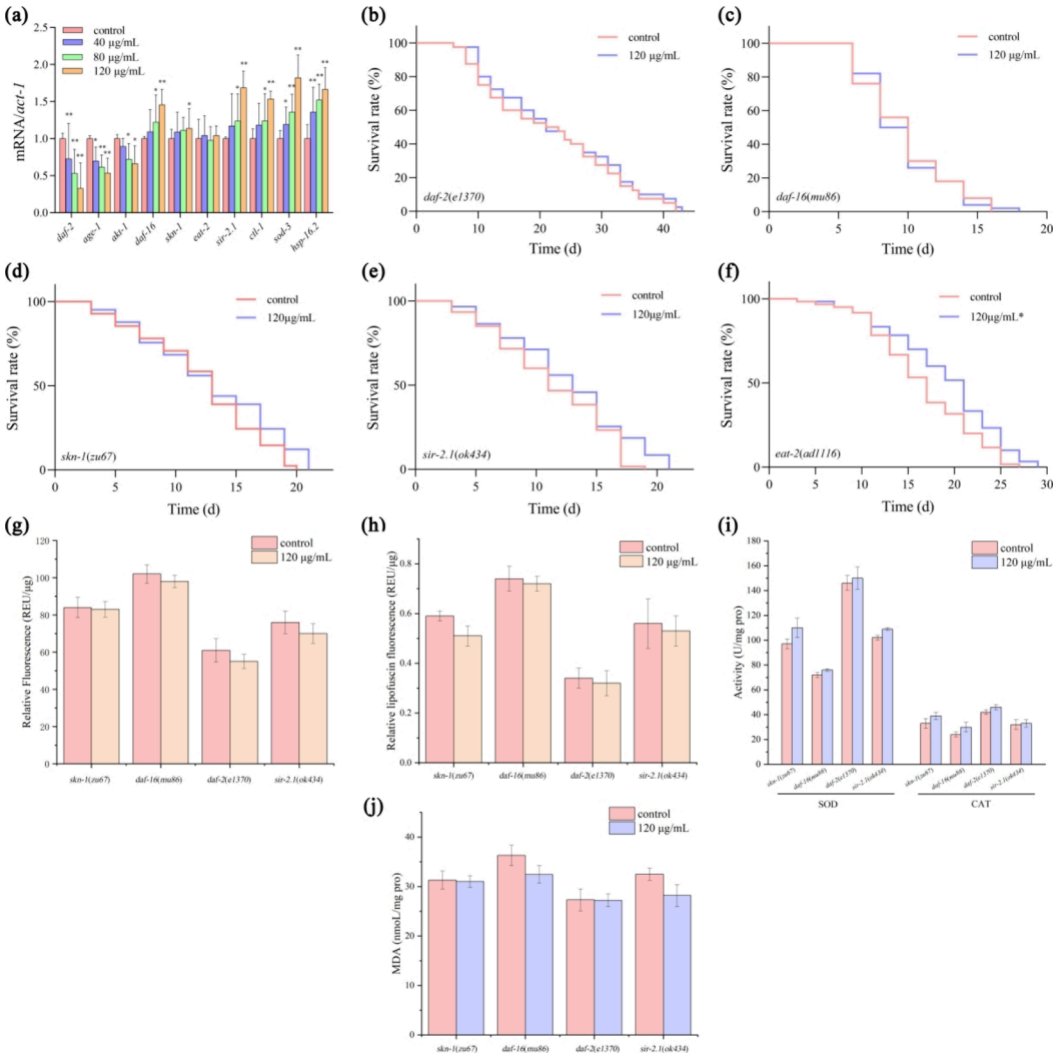


Figure 4. (a) Effects of phlorizin on the mRNA of longevity-related genes in *C. elegans*. Effect of phlorizin on the longevity of the mutant CB1370 *daf-2* (*e1370*) (b), CF1038 *daf-16* (*mu86*) (c), EU1 *skn-1* (*zu67*) (d), VC199 *sir-2.1* (*ok434*) (e), and DA1116 *eat-2* (*ad1116*) (f). The fluorescence quantification of ROS (g) and blue autofluorescence (h) in the mutant *C. elegans* with or without phlorizin supplementation. Effect of phlorizin on SOD and CAT activities (i) and MDA content (j) in the mutant *C. elegans*. $^{*}p < 0.05$ and $^{**}p < 0.01$.

Table 4. Lifespan of Different Genotypes of *C. elegans* Treated with Phlorizin

			lifespan (days)			
genotype	treatments (at 20 °C)	number	mean ± SD	median	extension % (mean)	log-rank test (*P* values)
N2	control	110	14.44 ± 1.60	15		
	40 μg/mL	109	15.56 ± 2.08	16	7.76	0.1707
	80 μg/mL	114	16.15 ± 1.30	16	11.84	0.0309[a]
	120 μg/mL	115	17.30 ± 2.01	18	19.81	0.0024[b]
daf-2 (*e1370*)	control	104	21.68 ± 0.71	22		
	120 μg/mL	117	22.88 ± 2.60	21	5.54	0.5423
daf-16 (*mu86*)	control	111	9.76 ± 2.33	10		
	120 μg/mL	106	9.64 ± 0.34	10	−1.23	0.8147
sir-2.1 (*ok434*)	control	104	11.40 ± 2.01	11		
	120 μg/mL	111	12.73 ± 1.72	13	11.67	0.0615
skn-1 (*zu67*)	control	115	12.29 ± 1.24	13		
	120 μg/mL	115	13.05 ± 1.62	13	18.91	0.1634
eat-2 (*ad1116*)	control	109	16.67 ± 1.23	17		
	120 μg/mL	109	18.93 ± 0.52	21	13.56	0.0133[a]
mek-1 (*ks54*)	control	107	10.40 ± 0.17	11		
	120 μg/mL	116	11.97 ± 2.54	13	15.10	0.0196[a]

[a]$p < 0.05$. [b]$p < 0.01$ in comparison to the control; each trial was repeated at least three times.

https://doi.org/10.1021/acsomega.4c08725
ACS Omega 2025, 10, 11922−11934

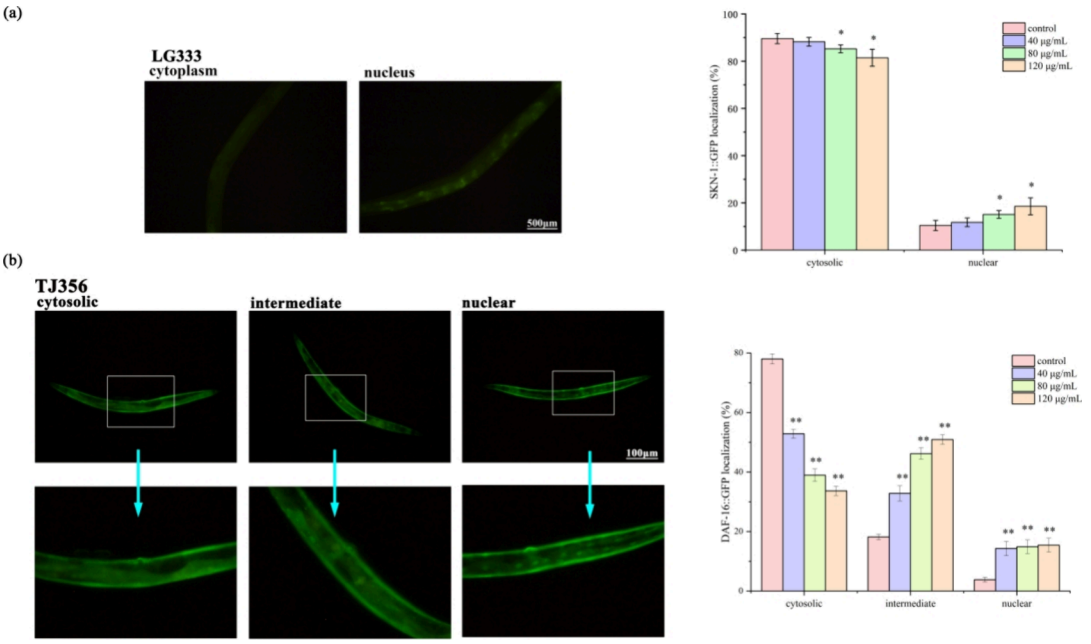


Figure 5. Effect of phlorizin on SKN-1 (a) and DAF-16 (b) nucleation and representative diagrams. *$p < 0.05$ and **$p < 0.01$.

correlation between oxidative stress and the onset of various age-related diseases, culminating in a decline in organismal health and lifespan. Phlorizin's multifaceted impact on physiological parameters is further underscored by its enhancement of antioxidant enzyme systems. Specifically, the observed increase in SOD and CAT activities, alongside a concomitant decrease in MDA content, suggests a bolstering of the endogenous antioxidant defenses (Figure 2e,f). These enzymes are pivotal in neutralizing ROS, thereby preventing the cascade of reactions leading to lipid peroxidation and cellular damage.[34] The modulation of these parameters by phlorizin is indicative of its potential to counteract the oxidative stress inherent to aging.[35] Moreover, the capacity of phlorizin to bolster resistance to hydrogen peroxide and high-temperature stress in *C. elegans* (Figure 2) is noteworthy. These environmental stressors are known to induce oxidative damage by generating hydroxyl radicals and disrupting metabolic homeostasis, respectively.[36] Phlorizin's ameliorative effects on these stress-induced injuries highlight its role in reinforcing the stress response mechanisms of the organism. This study collectively underscores the therapeutic potential of phlorizin in modulating the aging. Its ability to enhance antioxidant defenses, improve stress resistance, and attenuate the accumulation of oxidative by products positions phlorizin as a promising candidate for further research into interventions that may delay or ameliorate the aging process.

The integration of systems biology and network analysis within the realm of network pharmacology offers a sophisticated approach to dissecting the intricate dynamics between pharmacological agents and biological systems, with a particular emphasis on the analysis of drugs that target multiple biological pathways and the intricate regulation of signaling pathways.[37] Our investigation harnessed the predictive power of network pharmacology to elucidate the potential of phlorizin in modulating lifespan extension, primarily through the inhibition of the IIS pathway and the induction of a controlled oxidative stress response. Notably, the FoxO signaling pathway emerged as a pivotal player in the antiaging effects attributed to phlorizin, as depicted in Figure 3. The transcription factor DAF-16 in *C. elegans*, a functional counterpart to the mammalian FoxO family, plays a crucial role in mediating the longevity and stress resistance governed by the IIS pathway.[38] Our findings underscore the significance of phlorizin in augmenting the survival of *C. elegans* by modulating the expression of *daf-16* within the IIS pathway. Prior studies have established that mutations resulting in reduced *daf-2* activity can significantly amplify the lifespan of these organisms,[24] with the extent of this lifespan extension being intricately linked to the nuclear translocation of DAF-16.[39] In our study, we observed that phlorizin significantly upregulated the expression of the *daf-16* gene in the wild-type N2 strain, as illustrated in Figure 4a and detailed in Table S1. In contrast, the compound did not exert any discernible effect on the *daf-2* (*e1370*) mutant strain. These observations collectively suggest that the life-extending effects of phlorizin are mediated through the intricate interplay of the DAF-2/DAF-16 signaling axis, as schematically represented in Figure 6a. This study not only corroborates the role of the IIS pathway in modulating the lifespan but also expands our understanding of the molecular underpinnings of aging by identifying phlorizin as a novel modulator of this pathway. The implications of these findings extend beyond basic biological research, offering potential avenues for therapeutic intervention in age-related diseases and the promotion of healthy aging.

SIR-2.1 is an important member of the sirtuin family, which plays a key role in senescence regulation by directly acting on the *daf-16* gene through NAD-dependent deacetylation of the protein.[40] This mechanism is critical for lifespan regulation, particularly through the control of stress resistance conferred by the DAF-16 transcription factor.[41] In addition, previous studies have found that phlorizin can extend yeast lifespan by increasing the activity of endogenous antioxidant enzymes, including SOD, CAT, and Sir2,[12] but the specific regulatory mechanism was not indicated. Our study deepens our understanding of this issue by showing that phlorizin, a compound extracted from apples, can, at least to some extent,

https://doi.org/10.1021/acsomega.4c08725
ACS Omega 2025, 10, 11922−11934

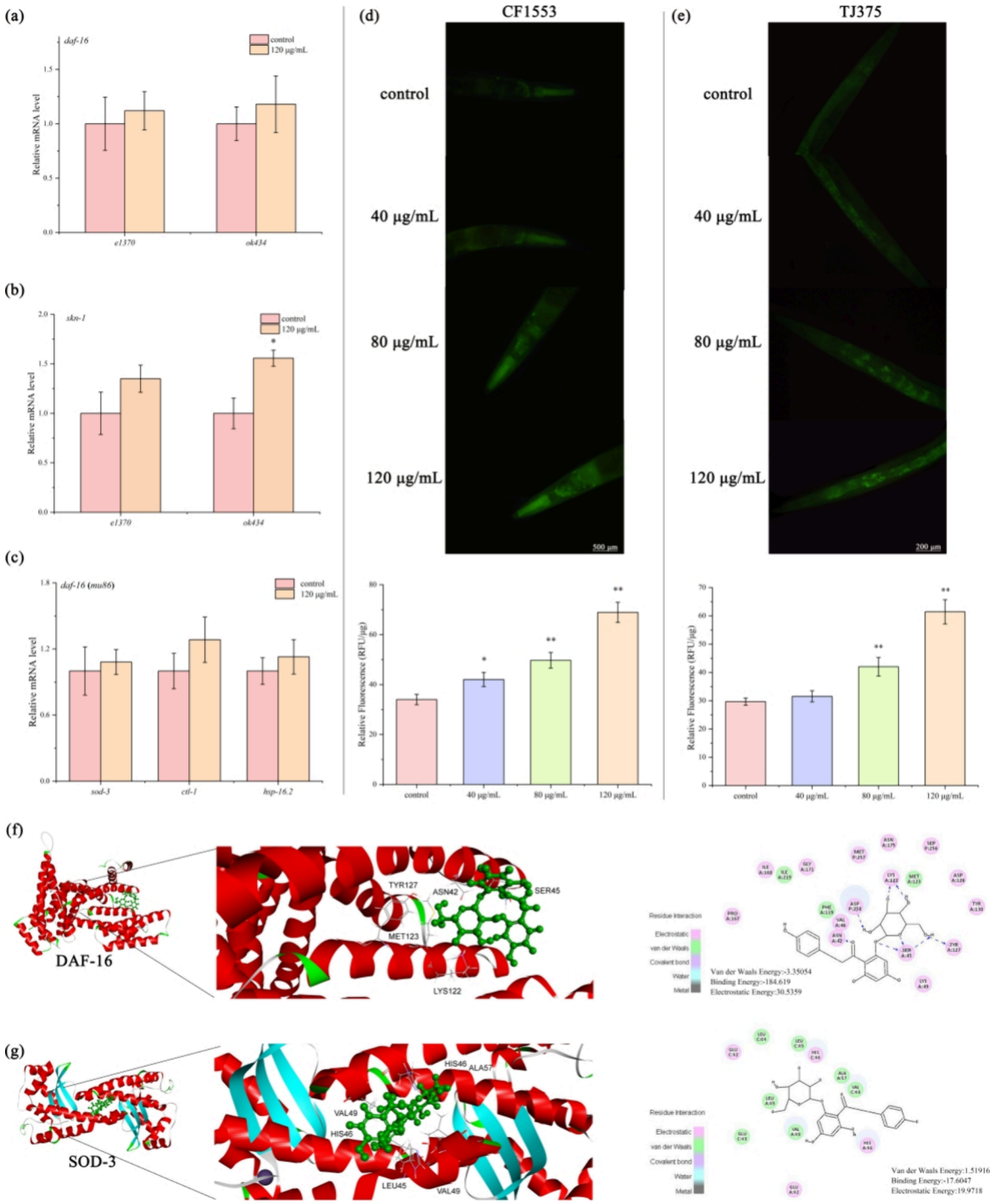


Figure 6. (a) Effect of phlorizin on the expression of *daf-16* in mutant CB1370 *daf-2* (*e1370*) and mutant VC199 *sir-2.1* (*ok434*). (b) Effect of phlorizin on the expression of skn-1 in mutant CB1370 *daf-2* (*e1370*) and mutant VC199 *sir-2.1* (*ok434*). (c) Effect of phlorizin on *sod-3*, *ctl-1*, and *hsp-16.2* expression in mutant CF1038 *daf-16* (*mu86*). (d) Effect of phlorizin on fluorescence quantification of SOD-3. (e) Effect of phlorizin on fluorescence quantification of HSP-16.2. Global detailed mapping of DAF-16 (f) and SOD-3 (g) proteins binding to phlorizin and their active site actions.

modulate the SIR-2.1 pathway to improve survival and antioxidant levels by defective nematodes and the antioxidant levels probed by phlorizin (Figure 4). In addition, the results of an assay to determine DAF-16 expression in defective nematodes showed that the effect of phlorizin on *daf-16* expression was dependent on SIR-2.1 activity (Figure 6a), which reinforces the intricate relationship between sirtuins and the IIS pathway in the regulation of senescence.[42]

The transcription factor SKN-1, similar to the mammalian Nrf2, is central to the cellular defense against oxidative stress.[43] Upon activation, it enters the nucleus to regulate genes involved in the antioxidant response.[44] Our research indicates that phlorizin activates Nrf2, thereby exerting antioxidant effects by inhibiting the Keap1-Nrf2 complex in *Drosophila melanogaster*.[12] Phlorizin has also been shown to reduce oxidative stress and improve cognitive and memory functions

https://doi.org/10.1021/acsomega.4c08725
ACS Omega 2025, 10, 11922−11934

Table 5. Molecular Docking of Phlorizin with the Receptor Proteins of *C. elegans*

ligand name	receptor name	interactive residues	hydrogen bonds involved residues	intermolecular energy (kcal mol^{-1})		binding energy (kcal mol^{-1})
				electrostatic energy	VDW energy	
phlorizin	DAF-16	PBO167, ILE168, GLY171, MET257, ASN175, SEP256, ASP126, TYR130, ASN42, VAL46, ASP258, LYS122, SER45, TYR127, LYS49, ILE219, PHE119, MET123	ASP258, ASN42, LYS122, SER45, TYR127	30.5359	−3.35054	−184.619
	SOD-3	GLUC42, GLU42, LEU45, VAL49(A), HIS46, GLU42,LEU64, LEU45, HIS46, ALA57,VAL49(C)	-	19.9718	1.51916	−17.6047

in aging mice through the Nrf2 pathway.[11] However, its impact on SKN-1 expression varies among species, as seen in the absence of effect in high-fat hamsters.[45] This variability in phlorizin's regulatory role emphasizes the need for a more nuanced understanding of its antioxidant mechanisms. Our findings reveal that phlorizin facilitates the nuclear translocation of SKN-1 (Figure 5a), potentially contributing to oxidative stress regulation and lifespan extension. This is in line with studies showing that the IIS pathway influences SKN-1 activity, affecting stress resistance and longevity.[46] In mutants, phlorizin's regulation of *skn-1* expression via the DAF-2 pathway, independent of SIR-2.1, suggests a dual mechanism for its antioxidant and antiaging effects (Figure 6b). This is consistent with Yoon et al.'s work on resveratrol's lifespan effects in *C. elegans*,[47] highlighting the conserved role of the IIS pathway in modulating longevity. In summary, our study sheds new light on phlorizin's complex interactions with molecular pathways like SIR-2.1/DAF-16 and SKN-1/Nrf2, suggesting its therapeutic potential in age-related diseases. Further research is essential to fully understanding its diverse mechanisms of action.

Dietary restriction (DR) is a well-established lifespan regulatory pathway, alongside the IIS pathway.[48] DR's impact on longevity is underscored by its ability to decrease Rpd3 activity, thereby enhancing dSir2 activity.[49] The transcription factor SKN-1, which plays a role in the DR response, is activated through the neuropeptide NRP-7.[50] Periodic DR has been shown to extend the lifespan of *D. melanogaster* by reducing insulin signal transcription.[51] The *eat-2* (*ad1116*) mutant, which exhibits decreased dietary intake, is considered a model for the effects of DR on longevity.[52] In our study, we found that phlorizin does not significantly regulate lifespan extension mainly through the DR pathway in *C. elegans*, which corroborates Yang's findings on otophylloside B's modulation of the IIS pathway independent of DR.[53] Taken together, the results reveal a key role for phlorizin in regulating DAF-16 mainly through IIS and SIR-2.1. Our research delves into the molecular mechanisms by which phlorizin influences the lifespan, particularly through its interaction with the DAF-16 pathway. We observed that phlorizin significantly promotes the nuclear localization of DAF-16 (Figure 5b) and activates the expression of DAF-16's downstream target genes, such as *sod-3*, *ctl-1*, and *hsp-16.2* (Figure 6c). These genes are crucial for the nematode's response to oxidative stress, with increased SOD-3 and HSP-16.2 expression effectively reducing ROS accumulation.[54,55] Phlorizin's pronounced effect on SOD-3 suggests that it primarily modulates *C. elegans* lifespan through the DAF-16 and SOD-3 axes.

Molecular coupling analyses were designed to probe the interaction of phlorizin with senescence-associated proteins, assessing the stability of the complexes by identifying binding energies and key interacting residues.[56] This study showed that in the optimal energy conformation, the arrangement of residues in the protein binding site favors the formation of hydrogen bonds with phlorizin. Calculation of the binding energies suggests that phlorizin can form stable complexes with key proteins such as DAF-16 and SOD-3, which may regulate senescence by influencing their function. Based on this, structural optimization of phlorizin can be performed to improve their binding affinity to these proteins. Despite the limitations of the current molecular docking and binding energy calculation methods, we plan to further validate the biological effects of phlorizin binding to DAF-16 and SOD-3 through in vivo animal model experiments and mutation experiments of key amino acid residues to inform the development of antiaging interventions. In conclusion, our study comprehensively reveals the role of phlorizin in life cycle regulation, highlighting its interactions with key molecular pathways.

5. CONCLUSIONS

This study comprehensively examines phlorizin's potential as an antiaging therapeutic, enhancing antioxidant defenses, modulating aging pathways, and improving stress resistance in *C. elegans*. Through transgenic models and molecular simulations, we uncover mechanisms influencing biomarkers such as ROS and blue autofluorescence while boosting antioxidant enzymes. The findings suggest that phlorizin could extend healthspan and delay the onset of age-related diseases, making it a promising candidate for clinical interventions. Moreover, by elucidating the involvement of key molecular pathways, such as IIS and SIR-2.1, with DAF-16 and SKN-1 as key targets, a foundation has been laid for the development of functional food additives for a wider audience.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c08725.

Statistical analysis of relative gene expression levels related to lifespan, effect of phlorizin on endogenous ROS accumulation in *C. elegans*, and effect of phlorizin on endogenous lipofuscin fluorescence accumulation in *C. elegans* (PDF)

Gene list analysis for protein-protein interactions using the STRING database (XLSX)

Protein−protein interaction (PPI) network (XLSX)

■ AUTHOR INFORMATION

Corresponding Author

Hao Wang − *State Key Laboratory of Food Nutrition and Safety, Tianjin University of Science and Technology*

https://doi.org/10.1021/acsomega.4c08725
ACS Omega 2025, 10, 11922−11934

(TUST), Tianjin 300457, China; School of Food Science and Technology, Dalian Polytechnic University, Dalian 116034, China; State Key Laboratory of Marine Food Processing and Safety Control, Dalian 116034, China; orcid.org/0000-0001-5975-506X; Phone: +86-13821138335; Email: wanghao@tust.edu.cn; Fax: +86-022-60601445

Author

Xiaohan Zhang − *State Key Laboratory of Food Nutrition and Safety, Tianjin University of Science and Technology (TUST), Tianjin 300457, China; School of Food Science and Technology, Dalian Polytechnic University, Dalian 116034, China; State Key Laboratory of Marine Food Processing and Safety Control, Dalian 116034, China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.4c08725

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the fund by Tianjin Science and technology project (17KPHDSF00120), the Natural Science Foundation of Tianjin (No. 18JCYBJC26500), and the "Novel Technology and relative mechanism of agricultural products processing and Storage" (TD13-5087).

■ REFERENCES

(1) Bhatiya, M.; Duttaroy, A. K.; Pathak, S.; Banerjee, A. Cellular Aging: An Introduction, Principle, Hallmarks, and Aging-Associated Diseases. In *Evidence-based Functional Foods for Prevention of Age-related Diseases*; Springer Nature Singapore: Singapore, 2023; pp 1−18.

(2) Sadiq, I. Z. Free Radicals and Oxidative Stress: Signaling Mechanisms, RedoxBasis for Human Diseases, and Cell Cycle Regulation. *CMM* **2023**, *23* (1), 13−35.

(3) Sachdev, S.; Ansari, S. A.; Ansari, M. I. Antioxidant Defensive Mechanisms to Regulate Cellular Redox Homeostatic Balance. In *Reactive Oxygen Species in Plants*; Springer Nature Singapore: Singapore, 2023; pp 143−172.

(4) Cheng, F.; Kotha, S.; Fu, M.; Yang, Q.; Wang, H.; He, W.; Mao, X. Nanozyme Enabled Protective Therapy for Neurological Diseases. *Nano Today* **2024**, *54*, No. 102142.

(5) Yun, H. M.; Hyun, B.; Song, X.; Hyun, S. Piwi Expressed in Drosophila Adipose Tissues Regulates Systemic IGF Signaling and Growth via IGF-Binding Protein. *Biochem. Biophys. Res. Commun.* **2024**, *695*, No. 149495.

(6) Zhou, C.; Zhou, Y.; Liang, Y.; Chen, L.; Liu, L.; Wei, F.; Li, G.; Duque, G. Fluoxetine Promotes Longevity via Reactive Oxygen Species in *Caenorhabditis Elegans*. *J. Gerontol.: Ser. A* **2024**, *79* (1), No. glad220.

(7) Xu, Y.; Miao, Y.; Li, R.; Manna, P. Nuciferine Promotes Longevity and Fitness in *Caenorhabditis Elegans* through the Regulation of the Insulin/IGF-1 Signaling Pathway. *J. Food Biochem.* **2024**, *2024* (1), 1.

(8) Shi, L.; Yu, X.-T.; Li, H.; Wu, G.-S.; Luo, H.-R. D-Chiro-Inositol Increases Antioxidant Capacity and Longevity of *Caenorhabditis Elegans* via Activating Nrf-2/SKN-1 and FOXO/DAF-16. *Experimental Gerontology* **2023**, *175*, No. 112145.

(9) Zhang, S.; Li, F.; Zhou, T.; Wang, G.; Li, Z. *Caenorhabditis Elegans* as a Useful Model for Studying Aging Mutations. *Front. Endocrinol.* **2020**, *11*, No. 554994.

(10) Abouelenein, D.; Caprioli, G.; Mustafa, A. M. Phloridzin: Advances on Resources, Biosynthetic Pathway, Bioavailability, Bioactivity, and Pharmacology. In *Handbook of Dietary Flavonoids*; Springer International Publishing: Cham, 2023; pp 1−29.

(11) Wang, H.; Sun, Z.; Liu, D.; Li, X.; Rehman, R.; Wang, H.; Wu, Z. Apple Phlorizin Attenuates Oxidative Stress in *Drosophila Melanogaster*. *J. Food Biochem* **2018**, No. e12744.

(12) Xiang, L.; Sun, K.; Lu, J.; Weng, Y.; Taoka, A.; Sakagami, Y.; Qi, J. Anti-Aging Effects of Phloridzin, an Apple Polyphenol, on Yeast *via* the SOD and Sir2 Genes. *Biosci., Biotechnol., Biochem.* **2011**, *75* (5), 854−858.

(13) Park, S.; Park, S.-K. Anti-Oxidant and Anti-Aging Effects of Phlorizin Are Mediated by DAF-16-Induced Stress Response and Autophagy in *Caenorhabditis Elegans*. *Antioxidants* **2022**, *11* (10), 1996.

(14) Ongay, K. K.; Granato, D.; Barreto, G. E. Comparison of Antioxidant Capacity and Network Pharmacology of Phloretin and Phlorizin against Neuroinflammation in Traumatic Brain Injury. *Molecules* **2023**, *28* (3), 919.

(15) Tian, Y.; Lu, W.; Deng, H.; Yang, F.; Guo, Y.; Gao, L.; Xu, Y. Phlorizin Administration Ameliorates Cognitive Deficits by Reducing Oxidative Stress, Tau Hyper-phosphorylation, and Neuroinflammation in a Rat Model of Alzheimer's Disease. *J. Food Biochem* **2018**, *42* (6), No. e12644.

(16) Dimmeler, S.; Nicotera, P. MicroRNAs in Age-related Diseases. *EMBO Mol. Med.* **2013**, *5* (2), 180−190.

(17) Chen, L.-H.; Chiou, G.-Y.; Chen, Y.-W.; Li, H.-Y.; Chiou, S.-H. microRNA and Aging: A Novel Modulator in Regulating the Aging Network. *Ageing Research Reviews* **2010**, *9*, S59−S66.

(18) Niu, Y.; DesMarais, T. L.; Tong, Z.; Yao, Y.; Costa, M. Oxidative Stress Alters Global Histone Modification and DNA Methylation. *Free Radical Biol. Med.* **2015**, *82*, 22−28.

(19) Cencioni, C.; Spallotta, F.; Martelli, F.; Valente, S.; Mai, A.; Zeiher, A.; Gaetano, C. Oxidative Stress and Epigenetic Regulation in Ageing and Age-Related Diseases. *IJMS* **2013**, *14* (9), 17643−17663.

(20) Lu, L.; Zhao, X.; Zhang, J.; Li, M.; Qi, Y.; Zhou, L. Calycosin Promotes Lifespan in *Caenorhabditis Elegans* through Insulin Signaling Pathway via *daf-16*, *age-1* and *daf-2*. *J. Biosci. Bioeng.* **2017**, *124* (1), 1−7.

(21) Schieber, M.; Chandel, N. S. TOR Signaling Couples Oxygen Sensing to Lifespan in *C. Elegans*. *Cell Reports* **2014**, *9* (1), 9−15.

(22) Bonuccelli, G.; Brooks, D. R.; Shepherd, S.; Sotgia, F.; Lisanti, M. P. Antibiotics That Target Mitochondria Extend Lifespan in *C. Elegans*. *Aging* **2023**, *15* (21), 11764−11781.

(23) Finkel, T.; Holbrook, N. J. Oxidants, Oxidative Stress and the Biology of Ageing. *Nature* **2000**, *408* (6809), 239−247.

(24) Zhang, J.; Lu, L.; Zhou, L. Oleanolic Acid Activates *daf-16* to Increase Lifespan in *Caenorhabditis Elegans*. *Biochem. Biophys. Res. Commun.* **2015**, *468* (4), 843−849.

(25) Bicca Obetine Baptista, F.; Arantes, L. P.; Machado, M. L.; Da Silva, A. F.; Marafiga Cordeiro, L.; Da Silveira, T. L.; Soares, F. A. A. Diphenyl Diselenide Protects a *Caenorhabditis Elegans* Model for Huntington's Disease by Activation of the Antioxidant Pathway and a Decrease in Protein Aggregation. *Metallomics* **2020**, *12* (7), 1142−1158.

(26) Spanoudakis, E.; Tavernarakis, N. Age-Associated Anatomical and Physiological Alterations in *Caenorhabditis Elegans*. *Mechanisms of Ageing and Development* **2023**, *213*, No. 111827.

(27) Chen, H.; Dong, L.; Chen, X.; Ding, C.; Hao, M.; Peng, X.; Zhang, Y.; Zhu, H.; Liu, W. Anti-Aging Effect of Phlorizin on D-Galactose−Induced Aging in Mice through Antioxidant and Anti-Inflammatory Activity, Prevention of Apoptosis, and Regulation of the Gut Microbiota. *Experimental Gerontology* **2022**, *163*, No. 111769.

(28) Coburn, C.; Allman, E.; Mahanti, P.; Benedetto, A.; Cabreiro, F.; Pincus, Z.; Matthijssens, F.; Araiz, C.; Mandel, A.; Vlachos, M.; Edwards, S.-A.; Fischer, G.; Davidson, A.; Pryor, R. E.; Stevens, A.; Slack, F. J.; Tavernarakis, N.; Braeckman, B. P.; Schroeder, F. C.; Nehrke, K.; Gems, D. Anthranilate Fluorescence Marks a Calcium-Propagated Necrotic Wave That Promotes Organismal Death in *C. Elegans*. *PLoS Biol.* **2013**, *11* (7), No. e1001613.

(29) Hernández-Cruz, E. Y.; Eugenio-Pérez, D.; Ramírez-Magaña, K. J.; Pedraza-Chaverri, J. Effects of Vegetal Extracts and Metabolites against Oxidative Stress and Associated Diseases: Studies in *Caenorhabditis Elegans*. *ACS Omega* **2023**, *8* (10), 8936−8959.
(30) Pincus, Z.; Mazer, T. C.; Slack, F. J. Autofluorescence as a Measure of Senescence in *C. Elegans*: Look to Red, Not Blue or Green. *Aging* **2016**, *8* (5), 889−898.
(31) Gerstbrein, B.; Stamatas, G.; Kollias, N.; Driscoll, M. *In Vivo* Spectrofluorimetry Reveals Endogenous Biomarkers That Report Healthspan and Dietary Restriction in *Caenorhabditis Elegans*. *Aging Cell* **2005**, *4* (3), 127−137.
(32) Garigan, D.; Hsu, A.-L.; Fraser, A. G.; Kamath, R. S.; Ahringer, J.; Kenyon, C. Genetic Analysis of Tissue Aging in *Caenorhabditis Elegans*: A Role for Heat-Shock Factor and Bacterial Proliferation. *Genetics* **2002**, *161* (3), 1101−1112.
(33) Eldred, G. E.; Miller, G. V.; Stark, W. S.; Feeney-Burns, L. Lipofuscin: Resolution of Discrepant Fluorescence Data. *Science* **1982**, *216* (4547), 757−759.
(34) Akpinar, D.; Mercan, T.; Demir, H.; Ozdemir, S.; Demir, C.; Kavak, S. Protective Effects of Thymoquinone on Doxorubicin-Induced Lipid Peroxidation and Antioxidant Enzyme Levels in Rat Peripheral Tissues. *PVJ.* **2023**, *43* (4), 651−658.
(35) Menezes, L. B.; Segat, B. B.; Tolentino, H.; Pires, D. C.; Mattos, L. M. D. M.; Hottum, H. M.; Pereira, M. D.; Latini, A.; Horn, A., Jr.; Fernandes, C. ROS Scavenging of SOD/CAT Mimics Probed by EPR and Reduction of Lipid Peroxidation in *S. Cerevisiae* and Mouse Liver, under Severe Hydroxyl Radical Stress Condition. *Journal of Inorganic Biochemistry* **2023**, *239*, No. 112062.
(36) Jomova, K.; Raptova, R.; Alomar, S. Y.; Alwasel, S. H.; Nepovimova, E.; Kuca, K.; Valko, M. Reactive Oxygen Species, Toxicity, Oxidative Stress, and Antioxidants: Chronic Diseases and Aging. *Arch. Toxicol.* **2023**, *97* (10), 2499−2574.
(37) Tian, F.; Yi, J.; Liu, Y.; Chen, B.; Wang, X.; Ouyang, Y.; Liu, J.; Tang, Y.; Long, H.; Liu, B. Integrating Network Pharmacology and Bioinformatics to Explore and Experimentally Verify the Regulatory Effect of Buyang Huanwu Decoction on Glycolysis and Angiogenesis after Cerebral Infarction. *Journal of Ethnopharmacology* **2024**, *319*, No. 117218.
(38) Todorova, M. N.; Savova, M. S.; Mihaylova, L. V.; Georgiev, M. I. Icariin Improves Stress Resistance and Extends Lifespan in *Caenorhabditis Elegans* through *hsf-1* and *daf-2*-Driven Hormesis. *IJMS* **2024**, *25* (1), 352.
(39) Okoro, N. O.; Odiba, A. S.; Han, J.; Osadebe, P. O.; Omeje, E. O.; Liao, G.; Liu, Y.; Jin, C.; Fang, W.; Liu, H.; Wang, B. *Ganoderma Lucidum* Methyl Ganoderate E Extends Lifespan and Modulates Aging-Related Indicators in *Caenorhabditis Elegans*. *Food Funct.* **2024**, *15* (2), 530−542.
(40) Vázquez-Lizarraga, R.; Mendoza-Viveros, L.; Cid-Castro, C.; Ruiz-Montoya, S.; Carreño-Vázquez, E.; Orozco-Solis, R. Hypothalamic Circuits and Aging: Keeping the Circadian Clock Updated. *Neural Regeneration Research* **2024**, *19* (9), 1919−1928.
(41) Garmendia-Berges, M.; Sola-Sevilla, N.; Mera-Delgado, Mc; Puerta, E. Age-Associated Changes of Sirtuin 2 Expression in CNS and the Periphery. *Biology* **2023**, *12* (12), 1476.
(42) Heidler, T.; Hartwig, K.; Daniel, H.; Wenzel, U. *Caenorhabditis Elegans* Lifespan Extension Caused by Treatment with an Orally Active ROS-Generator Is Dependent on DAF-16 and SIR-2.1. *Biogerontology* **2010**, *11* (2), 183−195.
(43) Tao, M.; Li, R.; Xu, T.; Zhang, Z.; Zheng, D.; Xia, Z.; Wu, T.; Pan, S.; Xu, X. Vitexin and Isovitexin Delayed Ageing and Enhanced Stress-Resistance through the Activation of the SKN-1/Nrf2 Signaling Pathway. *International Journal of Food Sciences and Nutrition* **2023**, *74* (6), 685−694.
(44) Gado, F.; Ferrario, G.; Della Vedova, L.; Zoanni, B.; Altomare, A.; Carini, M.; Aldini, G.; D'Amato, A.; Baron, G. Targeting Nrf2 and NF-κB Signaling Pathways in Cancer Prevention: The Role of Apple Phytochemicals. *Molecules* **2023**, *28* (3), 1356.
(45) Wang, H.; Sun, Z.; Rehman, R.-U.; Shen, T.; Riaz, S.; Li, X.; Hua, E.; Zhao, J. Apple Phlorizin Supplementation Attenuates Oxidative Stress in Hamsters Fed a High-Fat Diet. *J. Food Biochem* **2018**, *42* (1), No. e12445.
(46) Gómez-Linton, D. R.; Navarro-Ocaña, A.; Lobato-Ortiz, R.; Villa-Hernández, J. M.; Alavez, S.; Pérez-Flores, L. J. Effect of Tomato Extract on the Stress Resistance and Lifespan of *Caenorhabditis Elegans*. *Rev. Bras. Farmacogn.* **2022**, *32* (4), 645−650.
(47) Yoon, D. S.; Cha, D. S.; Choi, Y.; Lee, J. W.; Lee, M. MPK-1/ERK Is Required for the Full Activity of Resveratrol in Extended Lifespan and Reproduction. *Aging Cell* **2019**, *18* (1), No. e12867.
(48) Zhai, J.; Kongsberg, W. H.; Pan, Y.; Hao, C.; Wang, X.; Sun, J. Caloric Restriction Induced Epigenetic Effects on Aging. *Front. Cell Dev. Biol.* **2023**, *10*, 1079920.
(49) Kenyon, C. J. The Genetics of Ageing. *Nature* **2010**, *464* (7288), 504−512.
(50) Cypser, J. R.; Kitzenberg, D.; Park, S.-K. Dietary Restriction in C. Elegans: Recent Advances. *Experimental Gerontology* **2013**, *48* (10), 1014−1017.
(51) Romey-Glusing, R.; Li, Y.; Hoffmann, J.; Frieling, J.; Knop, M.; Pfefferkorn, R.; Bruchhaus, I.; Fink, C.; Roeder, T. Nutritional Regimens with Periodically Recurring Phases of Dietary Restriction Extend Lifespan in *Drosophila*. *FASEB j.* **2018**, *32* (4), 1993−2003.
(52) Cornwell, A. B.; Zhang, Y.; Thondamal, M.; Johnson, D. W.; Thakar, J.; Samuelson, A. V. *Elegans* Myc-Family of Transcription Factors Coordinate a Dynamic Adaptive Response to Dietary Restriction. *GeroScience* **2024**, *46* (5), 4827−4854.
(53) Motta, H. S.; Roos, D.; Tabarelli, G; Rodrigues, O. E. D.; Ávila, D.; Quines, C. B. Activation of SOD-3 Is Involved in the Antioxidant Effect of a New Class of β-Aryl-Chalcogenium Azide Compounds in *Caenorhabditis Elegans*. *An. Acad. Bras. Ciênc.* **2020**, *92* (suppl 2), No. e20181147.
(54) Zhao, S.; Cheng, Q.; Peng, Q.; Yu, X.; Yin, X.; Liang, M.; Ma, C. W.; Huang, Z.; Jia, W. Antioxidant Peptides Derived from the Hydrolyzate of Purple Sea Urchin (Strongylocentrotus Nudus) Gonad Alleviate Oxidative Stress in *Caenorhabditis Elegans*. *Journal of Functional Foods* **2018**, *48*, 594−604.
(55) Murphy, C. T.; McCarroll, S. A.; Bargmann, C. I.; Fraser, A.; Kamath, R. S.; Ahringer, J.; Li, H.; Kenyon, C. Genes That Act Downstream of DAF-16 to Influence the Lifespan of *Caenorhabditis Elegans*. *Nature* **2003**, *424* (6946), 277−283.
(56) Pagadala, N. S.; Syed, K.; Tuszynski, J. Software for molecular docking: a review. *Biophysical reviews* **2017**, *9* (2), 91−102.

https://doi.org/10.1021/acsomega.4c08725
ACS Omega 2025, 10, 11922−11934